How to build nanoblocks using DNA scaffolds

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Abstract. - In recent years there have been a number of proposals to utilize the specificity of DNA based interactions for potential applications in nanoscience. One interesting direction is the self-assembly of micro- and nanoparticle clusters using DNA scaffolds. In this letter we consider a DNA scaffold method to self-assemble clusters of "colored" particles. Stable clusters of identical microspheres have recently been produced by an entirely different method. Our DNA based approach self-assembles clusters with additional degrees of freedom associated with particle permutation. We demonstrate that in the non-equilibrium regime of irreversible binding the self-assembly process is experimentally feasible. These color degrees of freedom may allow for more diverse intercluster interactions essential for hierarchical self-assembly of larger structures.

DNA has attracted significant attention for its potential applications in nanoscience ([1], [2], [3], [4], [5], [6], [7], [8], [9], [10]). One recent non-DNA based advance is the self-assembly of stable clusters composed of identical microspheres [11]. In this letter we consider the self-assembly of micro- and nanoparticle clusters similar to those of [11], where DNA scaffolds govern the self-assembly process. The plan for the letter is the following. We first introduce the basic strategy of our self-assembly proposal. The goal is to maximize the yield for a particular type of cluster we call the star cluster. We analytically calculate the yield of the star cluster in the regime of irreversible binding. The analytical results are compared to the numerical results for the full aggregation equations. From an experimental perspective, the most important result is the determination of an optimal concentration ratio for experiments (see Eq. 8). To conclude we discuss the experimental feasibility of the self-assembly proposal.

The basic idea behind the procedure is as follows (see Fig. 1). Particles are functionalized with single-stranded DNA (ssDNA) markers which determine the particle color. There may be many DNA attached to each particle, but on any given particle the marker sequence is identical. One then introduces DNA scaffolds to the system. The scaffold is a structure with f ssDNA markers, each marker complementary to one of the particle colors. Hybridization of the ssDNA markers on the particles to those on the scaffold results in the formation of colored particle clusters. Because there are many DNA attached to each particle, clusters can form which contain more than one scaffold. The essential goal of the procedure is to maximize the concentration of a particular type of cluster which we denote the star cluster. The star cluster contains one and only one scaffold to which f particles are attached, each particle having a distinct color.

We should note that the role of the scaffold could also be played by a patchy particle ([12], [13], [14]). For example, these patches are regions on the particle surface where one can graft ssDNA markers. In this case there may be several DNA connections between a patch and colored particle. Our conclusions will still be valid, provided the patch size is chosen so that a patch interacts with at most one particle.

Previously we performed an equilibrium calculation to determine the yield of the star cluster [15]. The results of that study indicated that the concentration of scaffolds must be kept very small to prevent the aggregation of larger clusters. From an experimental perspective this result is somewhat disappointing, since the overall yield of the star cluster is proportional to the scaffold concentration. The situation is considerably improved in the regime of irreversible binding of particles to scaffolds. In what follows we present a calculation for the yield of the star cluster far from equilibrium.

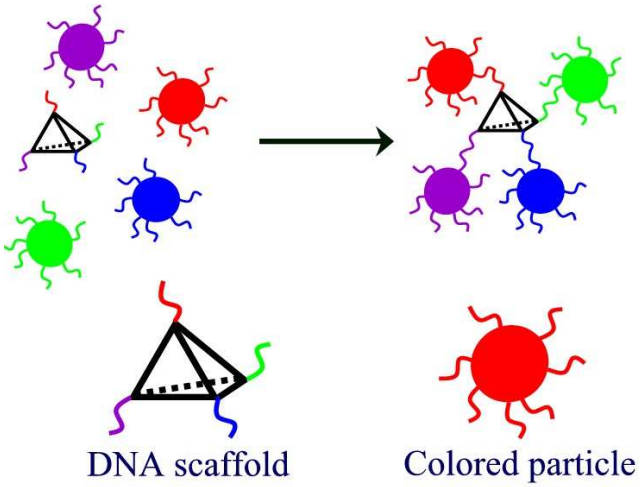

Fig. 1: **DNA scaffolding**. A graphical depiction of the scheme for self-assembling star clusters using DNA scaffolds. In the diagram (not drawn to scale) the scaffold funcionality $f = 4$.

To understand the basic physics behind the aggregation process we consider the mobility mismatch between the particles and the scaffolds. In solution, a particle with radius $R \simeq 1\mu m$ has a diffusion coefficient given by the Stokes-Einstein relation $D = k_B T/6\pi\eta R$. On the other hand, the size of the scaffold $a \simeq 10nm$. As a result the scaffolds diffuse $R/a \simeq 100$ times faster than the particles. To first approximation the resulting aggregation is a two stage process. In the first stage the particles recruit different numbers of scaffolds via the fast scaffold diffusion and subsequent DNA hybridization. Since we consider the regime of strong binding where these bonds are irreversible, the result is a Poisson distribution over the concentration of particles with m scaffolds attached. Let C_i denote the concentration of particles with color i, and c denote the total concentration of scaffolds. The total particle concentration $C_{tot} = \sum_{i=1}^{f} C_i$. The concentration $C_i^{(m)}$ of particles of color i with m scaffolds attached is

$$C_i^{(m)} = C_i \frac{p^m \exp(-p)}{m!} \quad (1)$$

$$p = \frac{c}{C_{tot}} \quad (2)$$

In the second stage there are no free scaffolds left in solution, and these particles decorated with scaffolds aggregate to form the final clusters. The seed to build a star cluster is a particle of any color with exactly one scaffold attached. This seed must aggregate with $f-1$ particles of different colors, each of which has no scaffolds. We now calculate the concentration of the star cluster C_*. The yield of the desired star cluster is quantified in terms of the star mass fraction $M_* = (fC_*)/C_{tot}$.

$$M_* = \frac{f}{C_{tot}} \sum_{i=1}^{f} C_i^{(1)} \prod_{\substack{j=1 \\ j\neq i}}^{f} \frac{C_j^{(0)}}{C_j} = x\exp(-x) \quad (3)$$

Here $x = fp$ is the scaffold functionality f multiplied by the concentration ratio p. By choosing $p = 1/f$ the mass fraction attains a maximum of $\exp(-1) \simeq 0.37$! This result indicates that by selecting the appropriate scaffold concentration, in the nonequilibrium regime up to 37% of the particles will aggregate to form star clusters. This is a significant improvement over the situation in the equilibrium regime.

This treatment of the problem captures the physics of star cluster formation, but it does not account for the loss of star clusters due to aggregation. In particular, as long as there are scaffolds with markers available for hybridization, when these scaffolds encounter a star cluster they can aggregate to form a larger cluster. We now estimate how this aggregation effects the final concentration of star clusters.

Consider the beginning of the second stage in our aggregation process. There are no longer any free scaffolds in solution, but a scaffold can have up to $f-1$ DNA markers still available for hybridization. We would like to determine how the star cluster mass fraction $M_*(y)$ changes as a function of the fraction of saturated scaffolds y. Here a saturated scaffold has particles hybridized to all f of its DNA markers, and is therefore unreactive. If s is the expectation that a slot on the scaffold is filled, then the fraction of saturated scaffolds is $y = s^{f-1}$. The average number of open slots on a scaffold is $(f-1)(1-s)$. Consider filling an open slot on the scaffold. The probability that the particle which filled the slot was part of a star cluster is $M_*(y)$. The average rate $r(y)$ at which star clusters are lost to aggregation is then

$$r(y) = -M_*(y)\frac{d}{dy}\left[(f-1)(1-s)\right] = M_*(y)y^{-\alpha}. \quad (4)$$

Here the exponent $\alpha = (f-2)/(f-1)$. We can then construct a differential equation for M_* taking into account this loss due to aggregation.

$$\frac{dM_*}{dy} = \frac{dM_*^{(o)}}{dy} - xr(y) \quad (5)$$

In the absence of this loss term the result of the calculation should recover our previous result Eq. 3. This zeroth order approximation is just $M_*^{(o)}(y) = xy\exp(-xy)$ which gives the correct star cluster concentration once all of the scaffolds are saturated ($y = 1$). To simplify the analysis a bit we take $\alpha = 1$ which is an excellent approximation in the limit of large scaffold functionality f. This is an inhomogeneous first order differential equation which can be solved by introducing an integrating factor $u(y) = y^x$. The initial condition which must be satisfied is $M_*(0) = 0$.

We are interested in the final star mass fraction M_*, which is $M_*(y=1)$. The result is

$$\begin{aligned} M_* &= x\sum_{k=0}^{\infty}\frac{(-x)^k}{k!}\left[\frac{1}{x+k+1}-\frac{x}{x+k+2}\right] \quad (6)\\ &= x\exp(-x)+x^2E_{-x}(x)-x^{1-x}\Gamma(1+x) \end{aligned}$$

Here $\Gamma(x)$ is the gamma function and $E_\nu(x) = \int_1^\infty t^{-\nu}\exp(-xt)dt$ is the exponential integral of order ν.

We can perform a similar type of analysis in the case when there is only one particle color. In this case the f ssDNA markers on the scaffold all have identical sequences complementary to this color. It turns out that the result for the mass fraction is the same. Because the mass fraction is the same in both cases, we can gain insight into the behavior of the system with many colors by analyzing the much simpler one color system. To test our predictions, we numerically solved a system of differential equations which models the irreversible aggregation between particles (one color) and scaffolds.

$$\frac{dC_{IJ}}{dt} = \frac{1}{2}\sum_{\substack{i+i'=I\\ j+j'=J}} K_{iji'j'}C_{ij}C_{i'j'} - C_{IJ}\sum_{i,j}K_{ijIJ}C_{ij} \quad (7)$$

This equation is the Smoluchowski coagulation equation [16] adapted to our system. C_{ij} is the concentration of the cluster with i scaffolds and j particles. $K_{iji'j'}$ is the rate constant for the irreversible reaction $C_{ij}+C_{i'j'}\rightarrow C_{i+i'j+j'}$. We assume that the rates are diffusion limited in which case we can estimate the rate for any pair of clusters by $K_{iji'j'} = 4\pi D_s R_l$. The larger cluster with hydrodynamic radius $R_l \sim n_l^{1/3}$ plays the role of a sink. Here n_l is the number of particles in the larger cluster and $D_s = k_BT/6\pi\eta R_s$ is the diffusion constant for the smaller cluster. To simply matters we only consider tree like structures, i.e. we do not consider the formation of clusters with internal loops. We have truncated the set of equations by considering clusters with a maximum of 10 scaffolds.

By solving these equations we can determine the concentration of stars $C_* = C_{1f}$ in this notation and test the validity of our two stage ansatz. As indicated in Fig. 2, the result of our analytical calculation matches the results of the full numerical calculation up to an overall normalization factor of order unity. Several points are in order.

The optimal concentration ratio p for experiments is easily determined from $\frac{dM_*}{dx}=0$. The result is $x_{\max}\simeq 0.47$. For scaffolds of functionality f the concentration ratio should be chosen as:

$$p = \frac{0.47}{f}. \quad (8)$$

Note that the maximum attainable star cluster yield $M_*(x_{\max})\simeq 1/4$ does not decrease with increasing f. In

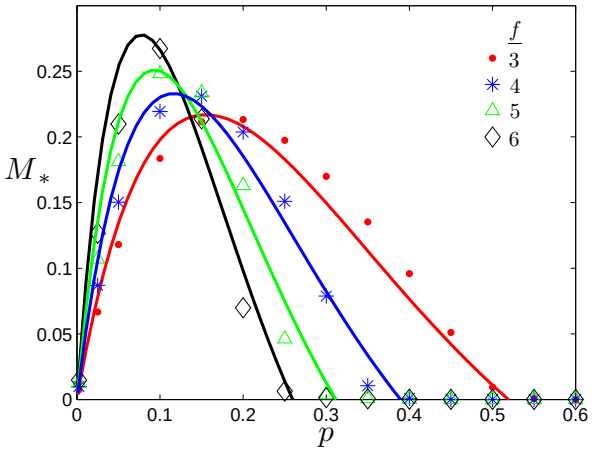

Fig. 2: **Star cluster mass fraction**. The mass fraction M_* as a function of p for scaffolds with functionality $f=3$ (red), 4 (blue), 5 (green), and 6 (black). The results determined numerically from the full solution of the Smoluchowski coagulation equation (markers) can be compared to results of the anlaytical calculation (lines) Eq. 6. The resulting agreement is good up to an overall normalization factor γ_f in the range 1.2 to 1.5 which normalizes the analytical curves.

fact, the numerical results predict a slight increase in star cluster yield for larger f. Solving the aggregation equations becomes computationally expensive, but it can still be done by reducing the maximum number of scaffolds in a cluster. For example, considering clusters with up to 5 scaffolds for $f=10$ gives $M_*(x_{\max})\simeq 0.3$. These results are important from the perspective of experimental feasibility for the self-assembly method. This is to be contrasted with the earlier equilibrium treatment. There the condition to suppress the aggregation of larger clusters imposed a fairly strict constraint [15] on the concentration ratio $p \lesssim f^{1/2}\left(\frac{2}{f}\right)^{f-1}$. From the perspective of self-assembling stars with large f this renders the regime of irreversible binding far more appealing than the equilibrium regime.

If an experiment is performed with the optimal concentration ratio, the clusters which self-assemble are easily separated by density gradient centrifugation [17]. In this regime most of the particles are monomers, in star clusters, or in saturated two scaffold clusters. These clusters contain, 1, f, and $2f-1$ particles respectively. The disparity in hydrodynamic radius and sedimentation velocity of these clusters makes the separation procedure experimentally feasible.

In this letter we considered a DNA scaffold method for self-assembling star clusters of f colored particles. By taking advantage of the mobility mismatch between particles and scaffolds, we were able to formulate a nonequilibrium calculation of the star mass fraction. The results of the calculation were compared to the numerical results of the full Smoluchowski coagulation equation for

the system. Good agreement is established between the analytical calculation and the numerics. In the regime of irreversible binding the yield of the desired star cluster is drastically improved in comparison to earlier equilibrium estimates. In nonequilibrium we find an experimentally feasible regime for the self-assembly of star clusters with a maximum mass fraction $\simeq 1/4$. We determined the optimal concentration ratio for an experimental implementation of our proposal. The additional color degrees of freedom associated with particle permutation in these clusters makes them ideal candidates as building blocks in a future hierarchical self-assembly scheme. In addition, these clusters can serve as the starting point to self-assemble structures of arbitrary geometry [18]. The experimental realization of self-assembling star clusters using DNA scaffolds would constitute an important step towards realizing the full potential of DNA mediated interactions in nanoscience.

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